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(54) Title: NOVEL LYSOPHOSPHATIDIC ACID ACYLTRANSFERASE

(57) Abstract

DNA and polypeptide sequences of a human lysophosphatidic acid acyltransferase (LPAAT) are disclosed. Methods and materials for production of LPAAT-1 and fragments and analogs thereof, production of antibodies, assays for identifying modulators of LPAAT and pharmaceutical compositions comprising LPAAT, polypeptides or modulators of LPAAT are provided. Also provided are methods for detecting LPAAT and lysophosphatidic acid.

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NOVEL LYSOPHOSPHATIDIC ACID ACYLTRANSFERASE

FIELD OF THE INVENTION

The present invention relates generally to the identification and isolation of a novel acyltransferase and more particularly to the discovery of a novel human lysophosphatidic acid acyltransferase.

Introduction

Lysophosphatidic acid (1-acyl-*sn*-glycero-3-phosphate, LPA) is a potent bioactive lipid with wide and diverse activities involved in physiologic and pathophysiologic biology. LPA is believed to be involved in natural physiologic functions including mitogenesis, cell differentiation, platelet aggregation, actin cytoskeleton remodeling, monocyte chemotaxis, smooth muscle contraction, and neurite retraction [Moolenaar, W.H., *J. Biol. Chem.*, *270*:12949-12952 (1995)]. In the Jurkat T-cell line LPA stimulates proliferation and IL-2 production suggesting that LPA is also involved in immune responses [Xu *et al.*, *J. Cell. Physiol.*, *163*:441-450 (1995a)]. The phospholipid may also participate in the pathophysiology of neurodegenerative processes by causing vasoconstriction as well as impairment of glutamate and glucose uptake by astrocytes [Tigyi *et al.*, *Am. J. Physiol, 268*:H2048-H2055, (1995); Keller *et al.*, *J. Neurochem, 67*:2300-2305 (1996)]. In addition, LPA is a potent promoter of tumor cell invasion [Imamura *et al.*, *Biochem. Biophys. Res. Comm, 193*:497-503 (1993)]. LPA exerts its biological effects via at least one, and perhaps multiple specific G protein-coupled receptors [van der Bend *et al.*, *EMBO J.*, *11*:2495-2501 (1992a); Hecht *et al.*, *J. Cell Biol.*, *135*:1071-1083 (1996), and Guo *et al.*, *Proc. Natl. Acad. Sci. USA, 93*:14367-14372 (1996)]. LPA binding to the G-protein coupled receptor results in activation of Ras and the Raf/MAP kinase pathway, stimulation of phospholipases C and D, inhibition of adenylyl cyclase, and tyrosine phosphorylation of focal adhesion proteins along with actin cytoskeleton remodeling [Moolenaar, *J. Biol. Chem.*, *270*:12949-12952 (1995)].

Because of the breadth of its biological impact, LPA metabolism has been a subject of intense study. During membrane phospholipid biosynthesis, LPA is formed by acylation of *sn*-glycerol-3-phosphate or by acylation of dihydroxyacetone phosphate (DHAP) followed by reduction of acyl-DHAP [Bishop and Bell, *Ann. Rev. Cell Biol.*, *4*:579-610 (1988)]. In contrast, LPA that is rapidly generated in the plasma membrane of thrombin-activated platelets and growth factor-stimulated fibroblasts [Fukumi and Takenawa, *J. Biol. Chem.*, *267*:10988-10993 (1992); Eichholtz *et al.*, *J. Biol. Chem.* *268*:1982-1986 (1993)] appears to be formed from hydrolysis of phosphatidic acid (PA) by a phospholipase A_2 [Gerrard and Robinson, *Biochim. Biophys. Acta*, *1001*:282-285 (1989); Billah *et al.*, *J. Biol. Chem.*, *256*:5399-5403 (1981); Thomson and Clark, *Biochem. J.*, *306*:305-309 (1995)]. Additionally, Fourcade and colleagues [Fourcade *et al.*, *Cell*, *80*:919-927 (1995)] have demonstrated that a secretory phospholipase A_2 acts upon membrane microvesicles shed from activated cells to convert PA to LPA. PA is a key intermediate in membrane phospholipid biosynthesis [Bishop and Bell, *Ann. Rev. Cell Biol.*, *4*:579-610 (1988)], but can also serve as a second messenger in activated cells [Agwu *et al.*, *J. Clin Invest.*, *88*:531-539 (1991)]. PA can be converted to CDP-diacylglycerol or to diacylglycerol by the action of PA phosphatase or back to LPA by the phospholipase A_2.

In normal serum, LPA is present at physiologically active concentrations. Because activated platelets copiously secrete the LPA, it has been suggested that aggregated platelets are the primary source of the serum LPA [Watson *et al.*, *Biochem. J*, *232*:61-66 (1985); Gerrard and Robinson, *Biochim. Biophys. Acta*, *1001*:282-285 (1989)]. The presence of LPA in serum, coupled with the mitogenic and chemotactic properties of LPA, suggests that the phospholipid is an important mediator of wound healing [Eichholtz *et al.*, *Biochem. J.*, *291*:677-680 (1993)]. Additionally, several of the known effects of LPA are consistent with a potential pro-inflammatory or pro-immune function. The fact that the LPA is present in serum at functional

concentrations implies the necessary presence of an "anti-LPA" mechanism to preclude inappropriate activation of LPA-sensitive cells. Consistent with this, there are at least three mechanisms whereby LPA bioactivity might be attenuated. First, LPA can be converted to PA in cells by the action of LPA acyltransferase (LPAAT) [Bishop and Bell, *Ann. Rev. Cell Biol., 4*:579-610 (1988); van der Bend *et al.*, *Biochim. Biophys. Acta, 1125*:110-112 (1992b)]. Second, Xie and Low, *Arch. Biochem. Biophys., 312*:254-259 (1994) described an *ecto*-(lyso) PA phosphatase that prefers as a substrate LPA or PA with a short *sn*-2 acyl chain. Finally, an LPA-specific lysophospholipase activity has been purified from rat brain [Thomson and Clark, *Biochem. J., 300*:457-461 (1994)].

Genetic and molecular biological approaches have facilitated cloning of genes encoding the LPAAT from non-mammalian species. However, no cloning of the mammalian counterparts of LPAAT has been reported.

In plants, storage triacylglycerols are synthesized via a four-step pathway that involves acylation of glycerol-3-phosphate at the *sn*-1 position to form LPA, acylation of LPA by LPAAT to form PA, then conversion of the PA to diacylgycerol by PA phosphatase followed by *sn*-3 acylation to form triacylglycerol [Browse and Somerville, *Annu. Rev. Plant Physiol. Plant Mol. Biol., 42*:467-506 (1991)]. Interest in this metabolic pathway with the goal of understanding and manipulating plant oil production has resulted in the cloning of several plant cDNAs that encode enzymes with LPAAT activity [Brown *et al.*, *Plant Mol. Biol., 26*:211-223 (1994), Brown *et al.*, *Plant Mol. Biol., 29*:267-278 (1995); Hanke *et al.*, *Eur. J. Biochem., 232*:806-810 (1995); Knutzon *et al.*, *Plant Physiol, 109*:999-1006 (1995)]. Interestingly, these cDNAs have extensive sequence homology with each other as well as with LPAAT cDNAs from prokaryotic organisms, yeast, and nematodes.

There thus continues to exist a need in the art for further insights into the nature, function and distribution of LPAATs providing means

for effecting beneficial modulation of these acyltransferases.

SUMMARY OF THE INVENTION

The present invention provides novel purified and isolated polynucleotides (*i.e.*, DNA and RNA both sense and antisense strands) encoding a human lysophosphatidic acid acyltransferase (LPAAT). LPAAT catalyzes the conversion of LPA to PA. LPA's biological functions, for example, include roles in mitogenesis, cell differentiation and platelet aggregation. LPA may also be involved in various disease states including neurodegenerative diseases and tumor cell invasion. LPAAT thus abrogates the activity of LPA by catalyzing its conversion to PA. Preferred LPAAT DNA sequences of the invention include genomic and cDNA sequences as well as wholly or partially chemically synthesized DNA sequences. The DNA sequence encoding LPAAT-1 that is set out in SEQ ID NO: 1 and DNA sequences which hybridize to a noncoding strand thereof under standard stringent conditions (or which would hybridize but for the redundancy of the genetic code) are contemplated by the invention. Exemplary stringent hybridization conditions are as follows: hybridization at 65°C in 3X SSC, 20mM $NaPO_4$ pH 6.8 and washing at 65°C in 0.2X SSC. It is understood by those skilled in the art that variation in these conditions occurs based on the length and GC nucleotide base content of the sequences to be hybridized. Formulas standard in the art are appropriate for determining exact hybridization conditions. See Sambrook *et al.*, 9.47-9.51 in *Molecular Cloning*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York (1989). DNA/DNA hybridization procedures carried out with DNA sequences of the invention under stringent conditions are expected to allow the isolation of DNAs encoding allelic variants of LPAAT-1; non-human species enzymes homologous to LPAAT-1; and other structurally related proteins sharing one or more of the enzymatic activities, or abilities to interact with members or regulators, of the cellular pathways in which LPAAT-1

participates.

Also contemplated by the invention are biological replicas (*i.e.*, copies of isolated DNA sequences made *in vivo* or *in vitro*) of DNA sequences of the invention. Autonomously replicating recombinant constructions such as plasmid and viral DNA vectors incorporating LPAAT sequences and especially vectors wherein DNA encoding LPAAT is operatively linked to an endogenous or exogenous expression control DNA sequence and a transcription terminator are also provided. The skilled worker understands the various components of vectors [*e.g.* promoter(s), selectable marker(s), origin of replication(s), multiple cloning site(s), etc.], methods for manipulating vectors and the uses of vectors in transforming or transfecting host cells (prokaryotic and eukaryotic) and expressing LPAAT of the present invention.

The DNA sequence information provided by the present invention also makes possible the development, by homologous recombination or "knockout" strategies [see *e.g.* Capecchi, *Science 244*:1288-1292 (1989)] of mammals that fail to express a functional LPAAT or that express a variant analog of LPAAT. The mammals of the present invention comprise a disrupted LPAAT gene or a disrupted homolog of the LPAAT gene. The general strategy utilized to produce the mammals of the present invention involves the preparation of a targeting construct comprising DNA sequences homologous to the endogenous gene to be disrupted. The targeting construct is then introduced into embryonic stem cells (ES cells) whereby it integrates into and disrupts the endogenous gene or homolog thereof. After selecting cells which include the desired disruption, the selected ES cells are implanted into an embryo at the blastocyst stage. Exemplary mammals include rabbits and rodent species.

Knowledge of DNA sequences encoding LPAAT-1 makes possible determination of the chromosomal location of LPAAT-1 coding sequences, as well as identification and isolation by DNA/DNA hybridization of genomic DNA sequences encoding the LPAAT-1 expression control regulatory

sequences such as promoters, operators, and the like. The chromosomal localization of these sequences may be useful in detection of inappropriate and/or over-expression of LPAAT-1 in various cell types.

The polynucleotides described herein are also useful for gene therapy. Gene therapy is described in U.S. Patent No. 5,399,346 hereby incorporated by reference. Briefly, gene therapy is the treatment of human diseases by transferring and expressing a gene encoding a therapeutic polypeptide in primary human cells. One aspect of this invention contemplates gene therapy utilizing LPAAT-1 encoding polynucleotides. Typically, the LPAAT-1 encoding polynucleotides are transferred to primary cells by viral vectors, through liposome mediated gene delivery or as naked DNA understood by the skilled worker. The genetically engineered primary cells are then introduced into the patient in need of gene therapy.

Also made available by the invention are antisense polynucleotides relevant to regulating expression of LPAAT by those cells which ordinarily express the same.

According to another aspect of the invention, prokaryotic or eukaryotic host cells are stably or transiently transformed with DNA sequences of the invention in a manner allowing the expression of LPAAT-1. Host cells expressing LPAAT-1 serve a variety of useful purposes. Such cells constitute a valuable source of immunogen for the development of antibody substances specifically immunoreactive with LPAAT-1. Host cells of the invention are also useful in methods for the large scale production of LPAAT-1 wherein the cells are grown in a suitable culture medium and the desired polypeptide products are isolated from the cells or from the medium in which the cells are grown by, for example, immunoaffinity purification.

Alternatively, host cells may be modified by activating an endogenous LPAAT-1 gene that is not normally expressed in the host cells or that is expressed at a lower rate than is desired. Such host cells are modified (*e.g.*, by homologous recombination) to express LPAAT-1 by replacing, in

whole or in part, the naturally-occurring LPAAT-1 promoter with part or all of a heterologous promoter so that the host cells express LPAAT-1. In such host cells, the heterologous promoter DNA is operatively linked to the LPAAT-1 coding sequences, *i.e.*, controls transcription of the LPAAT-1 coding sequences. See, for example, PCT International Publication No. WO 94/12650; PCT International Publication No. WO 92/20808; and PCT International Publication No. WO 91/09955. The invention also contemplates that, in addition to heterologous promoter DNA, amplifiable marker DNA (*e.g.*, ada, dhfr, and the multifunctional CAD gene which encodes carbamyl phosphate synthase, aspartate transcarbamylase, and dihydro-orotase) and/or intron DNA may be recombined along with the heterologous promoter DNA into the host cells. If linked to the LPAAT-1 coding sequences, amplification of the marker DNA by standard selection methods results in co-amplification of the LPAAT-1 coding sequences in such host cells.

As described herein, LPAAT-1 is an enzyme which possesses acyltransferase activity.

In one aspect, the present invention provides human LPAAT-1 polypeptides. Preferably, the human LPAAT-1 polypeptide sequences comprise the amino acid residues according to SEQ ID NO: 2.

The invention also contemplates polypeptide fragments and polypeptide analogs of LPAAT. As discussed in example 1 and in Figure 1, LPAAT-1 comprises four putative hydrophobic (transmembrane) domains and possibly four or five hydrophilic (cytosolic or extracellular) domains. LPAAT fragments comprising each of these domains or any combination thereof are an aspect of this invention. Alternatively, fragments comprising amino acid residues conserved among LPAATs (Figure 1) are contemplated. LPAAT analogs comprise additions, substitutions, including conservative substitutions, or deletions of amino acid residues which increase or decrease the acyltransferase activity of LPAAT, modify the solubility of LPAAT or an LPAAT fragment in aqueous and/or non-aqueous (*e.g.* liposomes) media.

The LPAATs of this invention (including fragments and analogs) may be modified to facilitate passage into the cell, such as by conjugation to a lipid soluble moiety. For example, LPAAT (or fragments or analogs thereof) may be conjugated to myristic acid. The LPAATs may be myristoylated by standard techniques as described in Eichholtz *et al.*, *Biochem. J.*, *291*:677-680 (1993), incorporated herein by reference. Alternatively, the LPAATs may be packaged in liposomes that may fuse with cell membranes and deliver the peptides into the cells. Encapsulation of the peptides in liposomes may also be performed by standard techniques as generally described in U.S. Patent Nos. 4,766,046; 5,169,637; 5,180,713; 5,185,154; 5,204,112; and 5,252,263 and PCT Patent Application No. 92/02244, each of which is incorporated herein by reference. Alternatively, LPAATs may be encapsulated in sterically stabilized liposomes (SSL). SSLs are liposomes wherein the lipids are covalently conjugated to water soluble (hydrophilic) polymers including polyethylene glycol and other well known polymers including for example, polyvinyl alcohol, polyglycolic acid, polyvinylpyrrolidone, and polyglycerol. It is believed that the presence of the hydrophilic polymer allows the SSL to remain in circulation longer than conventional liposomes thereby increasing the pharmacological efficacy of the encapsulated agent. SSLs are described in Lasic and Martin, Stealth Liposomes, CRC press, Inc., Boca Raton, FL (1995) which is hereby incorporated by reference.

Another aspect of this invention provides antibody substances (*e.g.*, polyclonal and monoclonal antibodies, antibody fragments, single chain antibodies, chimeric antibodies, CDR-grafted antibodies, humanized antibodies and the like) specifically immunoreactive with LPAAT. Antibody substances can be prepared by standard techniques using isolated naturally-occurring or recombinant LPAAT. The antibody substances are useful in modulating (*i.e.*, blocking, inhibiting, or stimulating) the acyltransferase activity of LPAAT. Antibody substances are also useful for purification of LPAAT and are also

useful for detecting and quantifying LPAAT in biological samples by known immunological procedures. In addition, cell lines (*e.g.*, hybridomas) or cell lines transformed with recombinant expression constructs which produce antibody substances of the invention are contemplated.

This invention further provides a method of detecting the presence of LPAAT-1 in a biological sample. The method comprises exposing an LPAAT specific antibody to a biological sample to be tested. The binding of the LPAAT specific antibody to LPAAT in the biological sample is detected by well-known means. For example, a second antibody conjugated to horseradish peroxidase (HRP) that specifically recognizes anti-LPAAT antibody is used to detect the presence of LPAAT. A positive color reaction catalyzed by HRP indicates that LPAAT is present in the biological sample.

Yet another aspect of this invention provides a method of detecting the presence of LPA in a biological sample. The presence of LPA is detected by exposing the biological sample to LPAAT and to a detectably labeled acyl donor (*e.g.*, radiolabeled acyl coenzyme A). The acyl transferase reaction is carried out under conditions similar to those described in Example 2 and the formation of detectably labeled PA is determined and quantitated. The amount of detectably labeled acyl chain transferred to the LPA present in the biological sample to form PA indicates the concentration of LPA. The generation of standard curves using known concentrations of LPA and LPAAT are understood by the skilled artisan.

In another aspect, methods of identifying a modulator that inhibits or stimulates the acyltransferase activity of LPAAT are contemplated. In a preferred method, the acyltransferase activity of LPAAT in the presence and absence of a potential modulator compound is determined and compared. A reduction in the acyltransferase activity observed in the presence of the test compound indicates that the test compound is an inhibitor. An increase in the acyltransferase activity observed in the presence of the test compound

indicates that the test compound is an activator. Modulators contemplated by the invention include organic and inorganic chemical compounds (including analogs of LPA and PA and polypeptides).

In addition, therapeutic/pharmaceutical compositions contemplated by the invention include LPAAT, a fragment or analog of LPAAT or a modulator of LPAAT and a physiologically acceptable diluent, carrier, or adjuvant and may also include other agents. In one aspect, dosage amounts indicated would be sufficient to supplement endogenous LPAAT activity and to inactivate pathological amounts of LPA. In another aspect, a sufficient dosage amount is that amount of LPAAT, fragment or analog of LPAAT or a modulator of LPAAT sufficient to supplement endogenous LPAAT activity. For general dosage considerations see *Remmington's Pharmaceutical Sciences*, 18th Edition, Mack Publishing Co., Easton, PA (1990). Dosages will vary between about 0.1 to about 1000 μg LPAAT or LPAAT modulator/kg body weight. Therapeutic compositions of the invention may be administered by various routes depending on the pathological condition to be treated. For example, administration may be by intravenous, subcutaneous, oral, suppository, topical and/or pulmonary routes.

Administration of LPAATs including LPAAT fragments or analogs thereof and modulators of LPAAT of the invention to mammalian subjects, especially humans, for the purpose of ameliorating pathological conditions is contemplated. LPAATs or LPAAT modulator compositions are useful in treating a mammal susceptible to or suffering from LPA-mediated pathological conditions including intracranial hemorrhage, tumorigenesis, fibrosis and restenosis. Such methods comprise administering the LPAAT modulator to the mammal in an amount sufficient to modulate LPAAT activity.

Numerous additional aspects and advantages of the present invention will be apparent from the following detailed description of illustrative embodiments thereof.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 shows a comparison of the amino acid sequences of human LPAAT-1 with LPAATs from the listed species. The predicted hydrophobic transmembrane domains of human LPAAT-1 are underlined. The amino acids conserved in all eight LPAATs are blocked.

Figure 2 shows bar graphs indicating the acyltransferase activity of recombinant human LPAAT-1 from transfected COS 7 cell lysates. The figure is further described in Example 2.

DETAILED DESCRIPTION OF THE INVENTION

The present invention is illustrated by the following examples. Example 1 describes the cloning and characterization of a cDNA encoding LPAAT-1. Example 2 describes the expression and acyltransferase activity of recombinant LPAAT-1. In Example 3, tissue expression patterns of LPAAT are described and in Example 4, the genomic structure and organization of LPAAT-1 is described. Example 5 discusses the role of LPAATs in various disease states.

Example 1

A TBLASTN search of the Genbank dbest database using the coconut LPAAT sequence [Knutzon, *et al., Plant Physiol.* 109:999-1006, (1995)] identified two human ESTs deposited having the accession numbers H39628 and H44282. Based upon the EST sequences, two oligonucleotide primers (Forward: 5'-GGG CCT CAT CAT GTA CCT CGG GGG CG-3' (SEQ ID NO: 3); Reverse: 5'-CTG CCC TCC CCC AGG TC-3' (SEQ ID NO: 4) were designed and used in polymerase chain reactions (PCR) to identify a clone (#82910123) in a human macrophage cDNA library [Tjoelker *et al.*, *Nature, 374*:549-553 (1995)] that contained sequence identical to the ESTs. The cDNA insert of clone #82910123 was used to generate a radiolabeled probe by random priming [Random Primed labeling kit

(Boehringer Mannheim, Indianapolis, IN)]. This probe was used to screen a human heart muscle cDNA library in Lambda Zap II and a genomic DNA library in Lambda Fix II (both from Stratagene, La Jolla, CA). Approximately $5x10^5$ to $1x10^6$ phage were blotted onto nitrocellulose and screened in 50% formamide, 0.75 M sodium chloride, 75 mM sodium citrate, 50 mM sodium phosphate (pH 6.5), 1% polyvinyl pyrolidine, 1% Ficoll, 1% bovine serum albumin (BSA), and 100 μg/ml sonicated salmon sperm DNA. After overnight hybridization at 42°C, blots were washed extensively in 3 mM sodium chloride, 0.3 mM sodium citrate, 0.1% SDS at 50°C. Following a secondary screen under identical conditions, individual hybridizing plaques were selected for DNA purification. The nucleotide sequence of both strands of the positively hybridizing heart cDNA clone 211-2 was determined.

The nucleotide sequence of LPAAT-1 (clone 211-2) obtained from the heart cDNA library comprises an open reading frame encoding a polypeptide of 278 amino acids with a predicted molecular mass of 30.9 kDa (Figure 1). The cDNA and the deduced amino acid sequences of human LPAAT-1 are provided in SEQ ID NOs.: 1 and 2, respectively. The predicted protein sequence exhibits approximately 23% identity with the coconut LPAAT but its identity with other members of the LPAAT family ranges up to approximately 33% (Table 1). While complete sequence identity at any given amino acid position between all members of the family is relatively infrequent, a core region of highly conserved amino acids is found from positions 167-205 of the human LPAAT-1 sequence (Figure 1).

Nucleotide sequences were analyzed with Geneworks (IntelliGenetics, Mountain View, CA). Amino acid sequence alignments were conducted using the ClustalW1 algorithm as found in the BCM Search Launcher - Multiple Sequence Alignments (http://dot.imgen.bcm.tmc.edu:9331/multi-align/multi-align.html).Individual pairwise alignments were conducted using Align Query (http://vega.crbm.cnrs-mop.fr/bin/align-guess.cgi). Transmembrane domain

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wound healing by its mitogenic effects on fibroblasts, smooth muscle cells and endothelial cells. However, in local or temporal excess, LPA may participate in propagating an inflammatory response. For example, LPA mediates platelet aggregation and monocyte chemotaxis [Moolenaar, W.H., *J. Biol. Chem.*, *270*:12949-12952 (1995)]. In addition, *in vitro* experiments suggest that LPA can also impact immune cell functions such as proliferation and IL-2 production (Xu *et al.*, *J. Cell. Physiol.*, *163*:441-450 (1995a)]. The requirement for physiological homeostasis predicts that there must be a mechanism to resolve the biological effects of LPA. One possible mechanism is to catabolize LPA via a phosphatase or lysophospholipase to produce a simple glycerolipid that is subject to rapid recycling into membrane phospholipids. In contrast, the product of LPA acylation by LPAAT is PA. A number of recent reports have suggested that PA may be a key intracellular messenger common to signalling pathways activated by pro-inflammatory mediators such as IL-1β, TNF-α, platelet-activating factor, and Lipid A [Bursten *et al.*, *J. Biol. Chem.*, *266*:20732-20743 (1991), Bursten *et al.*, *Am. J. Physiol.*, *262*:C328-C338 (1992), and Kester, M., *J. Cell. Physiol.*, *156*:317-325 (1993)] In these reports, it was demonstrated that PA was generated by the action of LPAAT.

As discussed above, LPA is a very potent mitogen, stimulating fibroblasts, smooth muscle cells, endothelial cells, keratinocytes, and early embryo cells to proliferate [Moolenaar, *J. Biol. Chem.*, *270*:12949-12952 (1995)]. There are a number of diseases, particularly of the lung, that are characterized by formation of fibrotic lesions that arise from extensive fibroblast proliferation. In conditions in which fibrosis can be anticipated (*e.g.*, acute respiratory distress syndrome, radiation therapy, aspiration pneumonia, chronic bronchitis, liver cirrhosis), administration of LPAAT, fragments or analogs of LPAAT or a modulator that increases the acyltransferase activity of LPAAT may serve to reduce fibroblast proliferation and the resultant fibrosis by converting LPA to PA.

Restenosis is a common outcome in angioplasty patients. The postulated mechanisms of restenosis include elastic recoil, smooth muscle cell proliferation with deposition of extracellular matrix, and remodeling (see Moreno *et al.*, *Circulation, 94(12)*:3098-3102 (1996) for review). Macrophages and smooth muscle cells are the primary cell types involved in the formation, progression and rupture of the atherosclerotic plaque. Given the mitogenic and chemotactic properties of LPA, administration of LPAAT, fragments or analogs of LPAAT or a modulator that increases the acyltransferase activity of LPAAT immediately before and in the weeks following an angioplasty procedure may reduce monocyte migration into the new lesion as well as limit smooth muscle cell proliferation.

LPA appears to play a role in brain physiology. The brain has the highest observed concentration of LPA [Das and Hajra, *Lipids, 24*:329-333 (1989)] and LPA receptors [van der Bend *et al.*, *EMBO J., 11*:2495-2501 (1992a); Hecht *et al.*, *J. Cell Biol., 135*:1071-1083 (1996)]. *In vitro* studies have demonstrated that a number of brain cell types, including cerebral cortical neurons, neuroblastomas, PC12 cells, and glial cells respond to LPA treatment. Keller, *et al.*, *J. Neurochem., 67*:2300-2305 (1996) demonstrated that LPA impaired glutamate uptake by astrocytes resulted in increased lipid peroxidation and decreased glucose uptake. Keller, *et al. J. Neurochem., 67*:2300-2305 (1996) noted that these effects can contribute to increased neuronal vulnerability during pathological conditions in which LPA levels are elevated.

An example of brain pathology in which LPA is elevated is described by Tigyi, *et al.*, *Am. J. Physiol., 268*:H2048-H2055 (1995). It was demonstrated that LPA is not normally present in cerebrospinal fluid. During intracranial hemorrhage, however, the concentration of LPA rapidly increased to very high levels. In this context, LPA functioned as a vasoconstrictor and inhibited vascular reactivity of cAMP by inhibiting adenylyl cyclase. Tigyi, *et al. Am. J. Physiol., 268*:H2048-H2055 (1995) concluded that platelet-

derived LPA may play an important role in the pathophysiology of altered vascular responsiveness seen after intracranial hemorrhage. Thus, administration of LPAAT activity or a modulator that increases LPAAT activity may reduce tissue damage occurring as a result of intracranial hemorrhage.

LPA has also been implicated in tumor biology. In an *in vitro* model of tumor cell invasion, Imamura *et al.*, *Biochem. Biophys. Res. Comm.*, *193*:497-503 (1993) demonstrated that certain tumor cell types (rat hepatoma and human small cell lung cancer) require LPA for penetration of endothelial or mesothelial cell layers. Furthermore, LPA was shown to stimulate calcium flux and proliferation in ovarian and breast cancer cell lines (Xu *et al.*, *Biochem. J.*, *309*:933-940 (1995b)]. Thus treatment with LPAAT, fragment or analog of LPAAT or a modulator that increases acyltransferase activity of LPAAT may prevent metastasizing tumors from invading healthy tissues by removal of LPA.

Alternatively, where there is systemic elevation of LPAAT, inhibition of LPAAT activity is indicated. Numerous reports have examined the biological consequences of preventing PA formation by inhibiting LPAAT. A small molecule inhibitor of the enzyme, lisofylline [(R)-1-(5-hydroxyhexyl)-3,7-dimethylxanthine] (LSF), blocks LPA metabolism and PA accumulation. Both *in vitro* and *in vivo* studies have demonstrated the anti-inflammatory properties of lisofylline. For example, Abraham *et al.*, *J. Exp. Med*, *181*:569-575 (1995) demonstrated that treatment with lisofylline prevented hypoxia-induced PA production as well as adherence and chemotaxis in human neutrophils. In cultured rat islet cells, IL-1β-induced cell dysfunction, measured by insulin secretion, was reduced in the presence of lisofylline [Bleich *et al.*, *Endocrinology, 137(11)*:4871-4877 (1996)]. *In vivo* studies further define the protective benefits of lisofylline: (1) Survival rates of 50-70% were achieved in mice treated with a lethal dose of endotoxin followed with intraperitoneal lisofylline administration [Rice *et al.*, *Proc. Natl. Acad.*

Sci. USA, 91:3857-3861 (1994)]. (2) Lisofylline blocked the development of interstitial lung edema and intra-alveolar hemorrhage, and reduced neutrophil accumulation in the lungs of mice subjected to hemorrhage and resuscitation. These effects may be due to the reduced TNF-α, IL-1β, and γIFN message levels found in mononuclear cells from the animals [Abraham *et al.*, *J. Exp. Med., 181*:569-575 (1995)]. (3) Pretreatment and one hour post-challenge treatment of septic pigs with lisofylline significantly reduced acute lung injury [Hasegawa *et al.*, *Am. J. Respir. Crit. Care Med., 155*:928-936 (1997)]. (4) Lisofylline reduced leakage of fluid into lungs of rats given IL-1 intratracheally, but did not affect neutrophil accumulation [Hybertson *et al.*, *J. Appl. Physiol., 82(1)*:226-232 (1997)]. Taken together, these data support the hypothesis that PA is an important pro-inflammatory intracellular messenger. In addition, many reports implicate PA as an extracellular agonist. The phospholipid purportedly stimulates monocyte migration [Zhou *et al.*, *J Biol. Chem., 270*:25549-25556 (1995)], is mitogenic to Balb-c/3T3 cells, causes superoxide generation in neutrophils, activates protein phosphorylation and stimulates phosphatidyl inositol-4-phosphate kinase, inactivates ras GTPase-activating protein and inhibits ras GTPase activity [reviewed in Martin *et al.*, *J. Biol. Chem., 268*:23924-23932 (1993)]. All of these observations suggest that PA is an important intercellular signaling molecule. Therefore, LPAAT may be involved in intercellular communication not only by its LPA metabolizing function but also by virtue of the PA it produces. These observations suggest that inhibition of LPAAT may be beneficial where there is systemic elevation of LPAAT under certain pathophysiological conditions.

The foregoing illustrative examples relate to presently preferred embodiments of the invention. Numerous modifications and variations thereof are expected to occur to those skilled in the art. Thus only such limitations as appear in the appended claims should be placed upon the scope of the present invention.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: ICOS Corporation

(ii) TITLE OF INVENTION: Novel Lysophosphatidic Acid Acyltransferase

(iii) NUMBER OF SEQUENCES: 19

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: Marshall, O'Toole, Gerstein, Murray & Borun
(B) STREET: 233 South Wacker Drive/6300 Sears Tower
(C) CITY: Chicago
(D) STATE: Illinois
(E) COUNTRY: United States of America
(F) ZIP: 60606

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Gass, David A.
(B) REGISTRATION NUMBER:38,153
(C) REFERENCE/DOCKET NUMBER: 27866/33878

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: (312)474-6300
(B) TELEFAX: (312)474-0448

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1639 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 184..1017

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

CGTAGGCTCC CTTCCCCTAC TCATCGCACT AATTTACACT CACAACACCC TAGGCTCACT 60

AAACATTCTA CTACTCACTC TCACTGCCCA AGAGCTATCA AACTCCCGGC CTGTGCGCGC 120

GGGGGAGAAG CGGGAGCGGG AGCGGGAGCG AGCTGGCGGC GCCGTCGGGC GCCGGGCCGG 180

```
GCC ATG GAG CTG TGG CCG TGT CTG GCC GCG GCG CTG CTG TTG CTG CTG        228
    Met Glu Leu Trp Pro Cys Leu Ala Ala Ala Leu Leu Leu Leu Leu
      1               5                  10                  15

CTG CTG GTG CAG CTG AGC CGC GCG GCC GAG TTC TAC GCC AAG GTC GCC        276
Leu Leu Val Gln Leu Ser Arg Ala Ala Glu Phe Tyr Ala Lys Val Ala
                20                  25                  30

CTG TAC TGC GCG CTG TGC TTC ACG GTG TCC GCC GTG GCC TCG CTC GTC        324
Leu Tyr Cys Ala Leu Cys Phe Thr Val Ser Ala Val Ala Ser Leu Val
            35                  40                  45

TGC CTG CTG CGC CAC GGC GGC CGG ACG GTG GAG AAC ATG AGC ATC ATC        372
Cys Leu Leu Arg His Gly Gly Arg Thr Val Glu Asn Met Ser Ile Ile
        50                  55                  60

GGC TGG TTC GTG CGA AGC TTC AAG TAC TTT TAC GGG CTC CGC TTC GAG        420
Gly Trp Phe Val Arg Ser Phe Lys Tyr Phe Tyr Gly Leu Arg Phe Glu
    65                  70                  75

GTG CGG GAC CCG CGC AGG CTG CAG GAG GCC CGT CCC TGT GTC ATC GTC        468
Val Arg Asp Pro Arg Arg Leu Gln Glu Ala Arg Pro Cys Val Ile Val
80                  85                  90                  95

TCC AAC CAC CAG AGC ATC CTG GAC ATG ATG GGC CTC ATG GAG GTC CTT        516
Ser Asn His Gln Ser Ile Leu Asp Met Met Gly Leu Met Glu Val Leu
                100                 105                 110

CCG GAG CGC TGC GTG CAG ATC GCC AAG CGG GAG CTG CTC TTC CTG GGG        564
Pro Glu Arg Cys Val Gln Ile Ala Lys Arg Glu Leu Leu Phe Leu Gly
            115                 120                 125

CCC GTG GGC CTC ATC ATG TAC CTC GGG GGC GTC TTC TTC ATC AAC CGG        612
Pro Val Gly Leu Ile Met Tyr Leu Gly Gly Val Phe Phe Ile Asn Arg
        130                 135                 140

CAG CGC TCT AGC ACT GCC ATG ACA GTG ATG GCC GAC CTG GGC GAG CGC        660
Gln Arg Ser Ser Thr Ala Met Thr Val Met Ala Asp Leu Gly Glu Arg
    145                 150                 155

ATG GTC AGG GAG AAC CTC AAA GTG TGG ATC TAT CCC GAG GGT ACT CGC        708
Met Val Arg Glu Asn Leu Lys Val Trp Ile Tyr Pro Glu Gly Thr Arg
160                 165                 170                 175

AAC GAC AAT GGG GAC CTG CTG CCT TTT AAG AAG GGC GCC TTC TAC CTG        756
Asn Asp Asn Gly Asp Leu Leu Pro Phe Lys Lys Gly Ala Phe Tyr Leu
                180                 185                 190

GCA GTC CAG GCA CAG GTG CCC ATC GTC CCC GTG GTG TAC TCT TCC TTC        804
Ala Val Gln Ala Gln Val Pro Ile Val Pro Val Val Tyr Ser Ser Phe
            195                 200                 205

TCC TCC TTC TAC AAC ACC AAG AAG AAG TTC TTC ACT TCA GGA ACA GTC        852
Ser Ser Phe Tyr Asn Thr Lys Lys Lys Phe Phe Thr Ser Gly Thr Val
        210                 215                 220

ACA GTG CAG GTG CTG GAA GCC ATC CCC ACC AGC GGC CTC ACT GCG GCG        900
Thr Val Gln Val Leu Glu Ala Ile Pro Thr Ser Gly Leu Thr Ala Ala
    225                 230                 235

GAC GTC CCT GCG CTC GTG GAC ACC TGC CAC CGG GCC ATG AGG ACC ACC        948
Asp Val Pro Ala Leu Val Asp Thr Cys His Arg Ala Met Arg Thr Thr
240                 245                 250                 255
```

```
TTC CTC CAC ATC TCC AAG ACC CCC CAG GAG AAC GGG GCC ACT GCG GGG        996
Phe Leu His Ile Ser Lys Thr Pro Gln Glu Asn Gly Ala Thr Ala Gly
                260                 265                 270

TCT GGC GTG CAG CCG GCC CAG TAGCCCAGAC CACGGCAGGG CATGACCTGG          1047
Ser Gly Val Gln Pro Ala Gln
            275

GGAGGGCAGG TGGAAGCCGA TGGCTGGAGG ATGGGCAGAG GGGACTCCTC CCGGCTTCCA     1107

AATACCACTC TGTCCGGCTC CCCCAGCTCT CACTCAGCCC GGGAAGCAGG AAGCCCCTTC     1167

TGTCACTGGT CTCAGACACA GGCCCCTGGT GTCCCCTGCA GGGGGCTCAG CTGGACCCTC     1227

CCCGGGCTCG AGGGCAGGGA CTCGCGCCCA CGGCACCTCT GGGAGCTGGG ATGATAAAGA     1287

TGAGGCTTGC GGCTGTGGCC CGCTGGTGGG CTGAGCCACA AGGCCCCCGA TGGCCCAGGA     1347

GCAGATGGGA GGACCCCGAG GCCAGACGCA CACTGTCCGA GCCCTCTGCT CAGCCGCCTG     1407

GGACCCACCA GGGTGCAGCT GGGCTCCAGG GTCCAGCCCA CAAGCTGCAT CAGGGTCTCT     1467

GGGAGAGGAG GGGCCTCCAG GGCCAGGAGT CCCAGACTCA CGCACCCTGG GCCACAGGGA     1527

GCCGGGAATC GGGGCCTGCT GCTCCTGCTG GCCTGGAAGA CTCTGTGGGG TCAGCACTGT     1587

ACTCCGTTGC TGTTTTTTTA TAAACACACT CTTGGAAGTG GAAAAAAAAA AA              1639
```

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 278 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

```
Met Glu Leu Trp Pro Cys Leu Ala Ala Ala Leu Leu Leu Leu Leu Leu
  1               5                  10                  15

Leu Val Gln Leu Ser Arg Ala Ala Glu Phe Tyr Ala Lys Val Ala Leu
             20                  25                  30

Tyr Cys Ala Leu Cys Phe Thr Val Ser Ala Val Ala Ser Leu Val Cys
         35                  40                  45

Leu Leu Arg His Gly Gly Arg Thr Val Glu Asn Met Ser Ile Ile Gly
     50                  55                  60

Trp Phe Val Arg Ser Phe Lys Tyr Phe Tyr Gly Leu Arg Phe Glu Val
 65                  70                  75                  80

Arg Asp Pro Arg Arg Leu Gln Glu Ala Arg Pro Cys Val Ile Val Ser
                 85                  90                  95

Asn His Gln Ser Ile Leu Asp Met Met Gly Leu Met Glu Val Leu Pro
            100                 105                 110

Glu Arg Cys Val Gln Ile Ala Lys Arg Glu Leu Leu Phe Leu Gly Pro
        115                 120                 125
```

Val Gly Leu Ile Met Tyr Leu Gly Gly Val Phe Phe Ile Asn Arg Gln
130 135 140

Arg Ser Ser Thr Ala Met Thr Val Met Ala Asp Leu Gly Glu Arg Met
145 150 155 160

Val Arg Glu Asn Leu Lys Val Trp Ile Tyr Pro Glu Gly Thr Arg Asn
165 170 175

Asp Asn Gly Asp Leu Leu Pro Phe Lys Lys Gly Ala Phe Tyr Leu Ala
180 185 190

Val Gln Ala Gln Val Pro Ile Val Pro Val Val Tyr Ser Ser Phe Ser
195 200 205

Ser Phe Tyr Asn Thr Lys Lys Lys Phe Phe Thr Ser Gly Thr Val Thr
210 215 220

Val Gln Val Leu Glu Ala Ile Pro Thr Ser Gly Leu Thr Ala Ala Asp
225 230 235 240

Val Pro Ala Leu Val Asp Thr Cys His Arg Ala Met Arg Thr Thr Phe
245 250 255

Leu His Ile Ser Lys Thr Pro Gln Glu Asn Gly Ala Thr Ala Gly Ser
260 265 270

Gly Val Gln Pro Ala Gln
275

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "primer"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

GGGCCTCATC ATGTACCTCG GGGGCG 26

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "primer"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

CTGCCCTCCC CCAGGTC 17

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 39 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "primer"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

AAGAGAATTC GTGCCGTGCG AGGACGCAAC GTCGAGAAC 39

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 36 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "primer"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

AATATCTAGA AGCATGGAGT GCCCGGACTC TGTCAG 36

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 539 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "primer"

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 2..539

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

```
C TTG CGT CTA ATG CTG CTC CAC ATC AAA TAC CTG TAC GGG ATC CGA          46
  Leu Arg Leu Met Leu Leu His Ile Lys Tyr Leu Tyr Gly Ile Arg
    1               5                  10                  15

GTG GAG GTG CGA GGG GCT CAC CAC TTC CCT CCC TCG CAG CCC TAT GTT        94
Val Glu Val Arg Gly Ala His His Phe Pro Pro Ser Gln Pro Tyr Val
                20                  25                  30

GTT GTC TCC AAC CAC CAG AGC TCT CTC GAT CTG CTT GGG ATG ATG GAG       142
```

```
Val Val Ser Asn His Gln Ser Ser Leu Asp Leu Leu Gly Met Met Glu
            35                  40                  45

GTA CTG CCA GGC CGC TGT GTG CCC ATT GCC AAG CGC GAG CTA CTG TGG      190
Val Leu Pro Gly Arg Cys Val Pro Ile Ala Lys Arg Glu Leu Leu Trp
         50                  55                  60

GCT GGC TCT GCC GGG CTG GCC TGC TGG CTG GCA GGA GTC ATC TTC ATC      238
Ala Gly Ser Ala Gly Leu Ala Cys Trp Leu Ala Gly Val Ile Phe Ile
      65                  70                  75

GAC CGG AAG CGC ACG GGG GAT GCC ATC AGT GTC ATG TCT GAG GTC GCC      286
Asp Arg Lys Arg Thr Gly Asp Ala Ile Ser Val Met Ser Glu Val Ala
 80                  85                  90                  95

CAG ACC CTG CTC ACC CAG GAC GTG AGG GTC TGG GTG TTT CCT GAG GGA      334
Gln Thr Leu Leu Thr Gln Asp Val Arg Val Trp Val Phe Pro Glu Gly
                100                 105                 110

ACG AGA AAC CAC AAT GGC TCC ATG CTG CCC TTC AAA CGT GGC GCC TTC      382
Thr Arg Asn His Asn Gly Ser Met Leu Pro Phe Lys Arg Gly Ala Phe
            115                 120                 125

CAT CTT GCA GTG CAG GCC CAG GTT CCC ATT GTC CCC ATA GTC ATG TCC      430
His Leu Ala Val Gln Ala Gln Val Pro Ile Val Pro Ile Val Met Ser
        130                 135                 140

TCC TAC CAA GAC TTC TAC TGC AAG AAG GAG CGT CGC TTC ACC TCG GGA      478
Ser Tyr Gln Asp Phe Tyr Cys Lys Lys Glu Arg Arg Phe Thr Ser Gly
    145                 150                 155

CAA TGT CAG GTG CGG GTG CTG CCC CCA GTG CCC ACG GAA GGG CTG ACA      526
Gln Cys Gln Val Arg Val Leu Pro Pro Val Pro Thr Glu Gly Leu Thr
160                 165                 170                 175

CCA GAT GAC GTC  C                                                  539
Pro Asp Asp Val
```

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 179 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

```
Leu Arg Leu Met Leu Leu His Ile Lys Tyr Leu Tyr Gly Ile Arg Val
  1               5                  10                  15

Glu Val Arg Gly Ala His His Phe Pro Pro Ser Gln Pro Tyr Val Val
            20                  25                  30

Val Ser Asn His Gln Ser Ser Leu Asp Leu Leu Gly Met Met Glu Val
        35                  40                  45

Leu Pro Gly Arg Cys Val Pro Ile Ala Lys Arg Glu Leu Leu Trp Ala
     50                  55                  60

Gly Ser Ala Gly Leu Ala Cys Trp Leu Ala Gly Val Ile Phe Ile Asp
 65                  70                  75                  80
```

```
Arg Lys Arg Thr Gly Asp Ala Ile Ser Val Met Ser Glu Val Ala Gln
                 85                  90                  95

Thr Leu Leu Thr Gln Asp Val Arg Val Trp Val Phe Pro Glu Gly Thr
            100                 105                 110

Arg Asn His Asn Gly Ser Met Leu Pro Phe Lys Arg Gly Ala Phe His
        115                 120                 125

Leu Ala Val Gln Ala Gln Val Pro Ile Val Pro Ile Val Met Ser Ser
    130                 135                 140

Tyr Gln Asp Phe Tyr Cys Lys Lys Glu Arg Arg Phe Thr Ser Gly Gln
145                 150                 155                 160

Cys Gln Val Arg Val Leu Pro Pro Val Pro Thr Glu Gly Leu Thr Pro
                165                 170                 175

Asp Asp Val
```

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 828 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..826

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

```
ATG CTG CTG CTG CTG CTC TTC CTG CTG CTG CTC TTC CTG CTG CCC ACC        48
Met Leu Leu Leu Leu Leu Phe Leu Leu Leu Leu Phe Leu Leu Pro Thr
1               5                   10                  15

CTG TGG TTC TGC AGC CCC AGT GCC AAG TAC TTC TTC AAG ATG GCC TTC        96
Leu Trp Phe Cys Ser Pro Ser Ala Lys Tyr Phe Phe Lys Met Ala Phe
            20                  25                  30

TAC AAT GGC TGG ATC CTC TTC CTG GCT GTG CTC GCC ATC CCT GTG TGT       144
Tyr Asn Gly Trp Ile Leu Phe Leu Ala Val Leu Ala Ile Pro Val Cys
        35                  40                  45

GCC GTG CGA GGA CGC AAC GTC GAG AAC ATG AAG ATC TTG CGT CTA ATG       192
Ala Val Arg Gly Arg Asn Val Glu Asn Met Lys Ile Leu Arg Leu Met
    50                  55                  60

CTG CTC CAC ATC AAA TAC CTG TAC GGG ATC CGA GTG GAG GTG CGA GGG       240
Leu Leu His Ile Lys Tyr Leu Tyr Gly Ile Arg Val Glu Val Arg Gly
65                  70                  75                  80

GCT CAC CAC TTC CCT CCC TCG CAG CCC TAT GTT GTT GTC TCC AAC CAC       288
Ala His His Phe Pro Pro Ser Gln Pro Tyr Val Val Val Ser Asn His
                85                  90                  95

CAG AGC TCT CTC GAT CTG CTT GGG ATG ATG GAG GTA CTG CCA GGC CGC       336
```

```
Gln Ser Ser Leu Asp Leu Leu Gly Met Met Glu Val Leu Pro Gly Arg
            100                 105                 110

TGT GTG CCC ATT GCC AAG CGC GAG CTA CTG TGG GCT GGC TCT GCC GGG      384
Cys Val Pro Ile Ala Lys Arg Glu Leu Leu Trp Ala Gly Ser Ala Gly
        115                 120                 125

CTG GCC TGC TGG CTG GCA GGA GTC ATC TTC ATC GAC CGG AAG CGC ACG      432
Leu Ala Cys Trp Leu Ala Gly Val Ile Phe Ile Asp Arg Lys Arg Thr
    130                 135                 140

GGG GAT GCC ATC AGT GTC ATG TCT GAG GTC GCC CAG ACC CTG CTC ACC      480
Gly Asp Ala Ile Ser Val Met Ser Glu Val Ala Gln Thr Leu Leu Thr
145                 150                 155                 160

CAG GAC GTG AGG GTC TGG GTG TTT CCT GAG GGA ACG AGA AAC CAC AAT      528
Gln Asp Val Arg Val Trp Val Phe Pro Glu Gly Thr Arg Asn His Asn
                165                 170                 175

GGC TCC ATG CTG CCC TTC AAA CGT GGC GCC TTC CAT CTT GCA GTG CAG      576
Gly Ser Met Leu Pro Phe Lys Arg Gly Ala Phe His Leu Ala Val Gln
            180                 185                 190

GCC CAG GTT CCC ATT GTC CCC ATA GTC ATG TCC TCC TAC CAA GAC TTC      624
Ala Gln Val Pro Ile Val Pro Ile Val Met Ser Ser Tyr Gln Asp Phe
        195                 200                 205

TAC TGC AAG AAG GAG CGT CGC TTC ACC TCG GGA CAA TGT CAG GTG CGG      672
Tyr Cys Lys Lys Glu Arg Arg Phe Thr Ser Gly Gln Cys Gln Val Arg
    210                 215                 220

GTG CTG CCC CCA GTG CCC ACG GAA GGG CTG ACA CCA GAT GAC GTC CCA      720
Val Leu Pro Pro Val Pro Thr Glu Gly Leu Thr Pro Asp Asp Val Pro
225                 230                 235                 240

GCT CTG GCT GAC AGA GTC CGG CAC TCC ATG CTC ACT GTT TTC CGG GAA      768
Ala Leu Ala Asp Arg Val Arg His Ser Met Leu Thr Val Phe Arg Glu
                245                 250                 255

ATC TCC ACT GAT GGC CGG GGT GGT GGT GAC TAT CTG AAG AAG CCT GGG      816
Ile Ser Thr Asp Gly Arg Gly Gly Gly Asp Tyr Leu Lys Lys Pro Gly
            260                 265                 270

GGC GGT GGG  T GA                                                    828
Gly Gly Gly
        275
```

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 275 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

```
Met Leu Leu Leu Leu Leu Phe Leu Leu Leu Leu Phe Leu Leu Pro Thr
  1               5                  10                  15

Leu Trp Phe Cys Ser Pro Ser Ala Lys Tyr Phe Phe Lys Met Ala Phe
            20                  25                  30
```

```
Tyr Asn Gly Trp Ile Leu Phe Leu Ala Val Leu Ala Ile Pro Val Cys
         35                  40                  45

Ala Val Arg Gly Arg Asn Val Glu Asn Met Lys Ile Leu Arg Leu Met
     50                  55                  60

Leu Leu His Ile Lys Tyr Leu Tyr Gly Ile Arg Val Glu Val Arg Gly
 65                  70                  75                  80

Ala His His Phe Pro Pro Ser Gln Pro Tyr Val Val Val Ser Asn His
                 85                  90                  95

Gln Ser Ser Leu Asp Leu Leu Gly Met Met Glu Val Leu Pro Gly Arg
            100                 105                 110

Cys Val Pro Ile Ala Lys Arg Glu Leu Leu Trp Ala Gly Ser Ala Gly
        115                 120                 125

Leu Ala Cys Trp Leu Ala Gly Val Ile Phe Ile Asp Arg Lys Arg Thr
    130                 135                 140

Gly Asp Ala Ile Ser Val Met Ser Glu Val Ala Gln Thr Leu Leu Thr
145                 150                 155                 160

Gln Asp Val Arg Val Trp Val Phe Pro Glu Gly Thr Arg Asn His Asn
                165                 170                 175

Gly Ser Met Leu Pro Phe Lys Arg Gly Ala Phe His Leu Ala Val Gln
            180                 185                 190

Ala Gln Val Pro Ile Val Pro Ile Val Met Ser Ser Tyr Gln Asp Phe
        195                 200                 205

Tyr Cys Lys Lys Glu Arg Arg Phe Thr Ser Gly Gln Cys Gln Val Arg
    210                 215                 220

Val Leu Pro Pro Val Pro Thr Glu Gly Leu Thr Pro Asp Asp Val Pro
225                 230                 235                 240

Ala Leu Ala Asp Arg Val Arg His Ser Met Leu Thr Val Phe Arg Glu
                245                 250                 255

Ile Ser Thr Asp Gly Arg Gly Gly Gly Asp Tyr Leu Lys Lys Pro Gly
            260                 265                 270

Gly Gly Gly
        275
```

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 42 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "primer"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

ATCAGAATTC CGGGAGCGGG AGCGGGAGCG AGCTGGCGGC GC 42

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 77 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "primer"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

ATTCTCTAGA CTACTTGTCA TCGTCGTCCT TGTAGTCCTG GGCCGGCTGC ACGCCAGACC 60

CCGCAGTGGC CCCGTTC 77

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 115 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: EXON 1
(B) LOCATION: 1..115

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

CGTAGGCTCC CTTCCCCTAC TCATCGCACT AATTTACACT CACAACACCC TAGGCTCACT 60

AAACATTCTA CTACTCACTC TCACTGCCCA AGAGCTATCA AACTCCCGGC CTGTG 115

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 572 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: EXON 2
(B) LOCATION: 46..295

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

CCCCGCCCCG CCCAGCCCCG CCGCCTTCGC AATAAGGGGC CTGAGCGCGC GGGGGAGAAG 60

CGGGAGCGGG AGCGGGAGCG AGCTGGCGGC GCCGTCGGGC GCCGGGCCGG GCCATGGAGC 120

TGTGGCCGTG TCTGGCCGCG GCGCTGCTGT TGCTGCTGCT GCTGGTGCAG CTGAGCCGCG 180

CGGCCGAGTT CTACGCCAAG GTCGCCCTGT ACTGCGCGCT GTGCTTCACG GTGTCCGCCG 240

TGGCCTCGCT CGTCTGCCTG CTGCGCCACG GCGGCCGGAC GGTGGAGAAC ATGAGGCAAG 300

GCCGGGGGCC GCCGGGAGGG GCCGGGGAAC CGCCGCGCCG CTTCCGCTTC CCTAACTTTC 360

TTCTGGGCTT CCCTCCTTCC TGCCCCGCCC GTCCCGCCCC GCTCCGGGGC TCCGGGGAGA 420

GCGCGCCTGG GCCGGCGGCA GGCACAGGAG GGGGTCCCGG AGTCAGGGGG TCCCGGAGTC 480

ACGGGGTCAA GGAGCCGGCG TCACAGTGCC CAGCACCCCA CCCCCCGCCC TGGCCCCGGG 540

CGTCTACACC GGTTTCGGCC TCCGCCGCGT CC 572

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 383 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: EXON 3
(B) LOCATION: 224..357

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

CTCTGTTGCT GGGGAGACGG AGGCAGGGCA GCCGTCCAGG TGGGTGAGCC GGGCCCGGGA 60

CTCTGTCCGC TTCAGGGGCT CCCTCCCCTG TGTCTCCCGG TCTCCTGCCC CGTGCCAGGA 120

GGGCCCCTCC CCAGCCTCCT CCACACCCCA CCCCCAGGCC TTCCCGCCCC AGCCTCGGCT 180

GCGGGATCTG TGGGACCCGT GTTCATGGTG GCCTCCCCTG CAGCATCATC GGCTGGTTCG 240

TGCGAAGCTT CAAGTACTTT TACGGGCTCC GCTTCGAGGT GCGGGACCCG CGCAGGCTGC 300

AGGAGGCCCG TCCCTGTGTC ATCGTCTCCA ACCACCAGAG CATCCTGGAC ATGATGGGTA 360

GGCCGGGCCT CGGGGTGGCT TCT 383

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 974 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: EXON 4
(B) LOCATION: 504..679

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

CCACACCCCA CCCGCAGGCT TTCCCGCCCC AGCTTGGGGT GCGGGATCTG TGGGACCGGT 60

GTTCATGGTG GCCTCCCCTG CAGCATCATC GGGTGGTTCG TGCGAAGCTT CAAGTACTTT 120

TACGGGCTCC GCTTCGAGGT GCGGGACCCG CGCAGGCTGC AGGAGGCCCG TCCCTGTGTC 180

ATGGTCTCCA ACCACCAGAG CATCCTGGAC ATGATGGGTA GGCCGGGCCT CGGGGGTGGC 240

TTCTGGGGTT TGAGTGGGGC CGGCTGAGCT GGGGCTGTGT GGGGCTGGGT CCCGGGGACG 300

AGGACACAGG GCTGCCTGTG CCTGGGCGAG CTCGGCCTCA GTACCTCCCT CAGGGCCAGA 360

CACAGAGGCT CGGAGGCCAC ACGACCCGTC CAGGTAGCCA GGGAGAAGGC AGGGTGCCAG 420

GCAGGCCTGT GGGTGCTCAG CAGCTGTCTT CCAGCGCACG CTGTCTCCCC CTCTCTCTCT 480

GTCTCTGTCT CTCTGTCTCC CAGGCCTCAT GGAGGTCCTT CCGGAGCGCT GCGTGCAGAT 540

CGCCAAGCGG GAGCTGCTCT TCCTGGGGCC CGTGGGCCTC ATCATGTACC TCGGGGGCGT 600

CTTCTTCATC AACCGGCAGC GCTCTAGCAC TGCCATGACA GTGATGGCCG ACCTGGGCGA 660

GCGCATGGTC AGGGAGAACG TGAGTTAGCA AGGCCGGGCT CGGTGGGGTT AGGGTGGGGC 720

CTAGGGCGGG GCCAAGCAGG GGCCAGCTTG TGACTTGGTT TTGGCACAAA AAACAAGACC 780

CCCACATCAT CCATGCTCCG CAGGTGGGGT CCCACGCCAG ACCCCTACAT CATCCATGGC 840

TCCGCATATG GGGTCCCATG CCAGCTGCTT TGCGAAATGG GGCTTCTTAA GAGGCGAGGC 900

GGTGTGGCCT TTCTGGGGTG GCCTGGGCGT GAGGTCAATC CAAGCTCTCC TCTCCCTGCA 960

GCTCAAAGTG TGGA 974

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 351 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: EXON 5
(B) LOCATION: 77..172

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

CTTAGGAGGC GAGGCGGCGT GGCCTTTCTG GGGTGGCCTG GGCGTGAGGT CAGTCCAGGC 60

TCTCCTCTCC CTGCAGCTCA AAGTGTGGAT CTATCCCGAG GGTACTCGCA ACGACAATGG 120

GGACCTGCTG CCTTTTAAGA AGGGCGCCTT CTACCTGGCA GTCCAGGCAC AGGTAGGCTG 180

AGCCCACCCC TCCCTGGCGT GGGTGCAGGC TGGGGAGGCG GGGTCAGGCT GGCTTAAGGC 240

AGCATGTGAC CACCACCCGA GCTGAGGACC CTTGACACAC AAGGGACTCC TCCCACTGAG 300

TTGGGGACAG GGCCTCCTTG CCCCTTCCTG CACTTGCCCC CTGACCGACC A 351

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 713 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: EXON 6
(B) LOCATION: 299..371

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

CTGGCTAGGT GCAGACCCAA TATGGGGACA GGTGTGAACC AGGCAAACAG GTGCTAGCTC 60

AGGAGGGCTT CCTAGAGGAG ATGAGTAAAA ATTTGCAGGA TGAGCTTATG GAACTGTGGG 120

CCAACCAGAG CAGGACACAT ATCCCAGGTC CCAGGGGCAG GACAGCCACA AGGACCCATG 180

GCAGCAGCGA AGGCAGGGGT GGGTGGGCCG CAGGACAGGG TTCCCCAACC ACATGCAGCC 240

TGGGGTGTGC CTGGCCTGTC CCCAGGGCTG CTTCAGCTGT GCGTCTCCCT GCCTGCAGGT 300

GCCCATCGTC CCCGTGGTGT ACTCTTCCTT CTCCTCCTTC TACAACACCA AGAAGAAGTT 360

CTTCACTTCA GGTACCCCCA CATGTGTGCA CCCGGGGTGT AGGCCCCGCC TGACCCTACA 420

GTCACGGAGC CCCGGGCCCC TCATCGTTCC CATTTCCGGG TGGCACCCGT GGCGTGGCCA 480

CACGGTGACC ACGTGGCGAA TGAGTGACTC ACGCTGGAGT CCCACCTGTG GGCTTCATGG 540

CCTCATGGCC CTTCCAGCCA GTTCCCAGAA CGTGGGCACC TGGTGCCCAC GCAGGACAGT 600

GGGGTCAAAG TTGGACAGCA GTGGGGGAAC CCACCTCCAT CTCTCCCACA GCCCCTCGCC 660

CCGTATGGAG GGCAGAGGCC ACGCAGTGAG GTACGGGCTG ATCAAGAACT GGG 713

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1088 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: EXON 7
(B) LOCATION: 141..924

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

AGGGAGTCCA GGGGAAGAGC CCGGCCTCGG GCTTCCCAGG GAGGGGCTGT GGGGGGCTGG 60

GGAGAGGCGA GGCCAGGGCA GCAGGCTGAG GTGGGCCCCA GCTCCCCACA GGCCACTGAG 120

GTCTGTTGCT TCCCCCACAG GAACAGTCAC AGTGCAGGTG CTGGAAGCCA TCCCCACCAG 180

CGGCCTCACT GCGGCGGACG TCCCTGCGCT CGTGGACACC TGCCACCGGG CCATGAGGAC 240

CACCTTCCTC CACATCTCCA AGACCCCCCA GGAGAACGGG GCCACTGCGG GGTCTGGCGT 300

GCAGCCGGCC CAGTAGCCCA GACCACGGCA GGGCATGACC TGGGGAGGGC AGGTGGAAGC 360

CGATGGCTGG AGGATGGGCA GAGGGGACTC CTCCCGGCTT CCAAATACCA CTCTGTCCGG 420

CTCCCCCAGC TCTCACTCAG CCCGGGAAGC AGGAAGCCCC TTCTGTCACT GGTCTCAGAC 480

ACAGGCCCCT GGTGTCCCCT GCAGGGGGCT CAGCTGGACC CTCCCCGGGC TCGAGGGCAG 540

GGACTCGCGC CCACGGCACC TCTGGGAGCT GGGATGATAA AGATGAGGCT TGCGGCTGTG 600

GCCCGCTGGT GGGCTGAGCC ACAAGGCCCC CGATGGCCCA GGAGCAGATG GGAGGACCCC 660

GAGGCCAGAC GCACACTGTC CGAGCCCTCT GCTCAGCCGC CTGGGACCCA CCAGGGTGCA 720

GCTGGGCTCC AGGGTCCAGC CCACAAGCTG CATCAGGGTC TCTGGGAGAG GAGGGGCCTG 780

GAGGGCCAGG AGTCCCAGAC TCACGCACCC TGGGCCACAG GGAGCCGGGA ATCGGGGCCT 840

GCTGCTCCTG CTGGCCTGGA AGACTCTGTG GGGTCAGCAC TGTACTCCGT TGCTGTTTTT 900

TTATAAACAC ACTCTTGGAA GTGGCTGGGG AGCTGTGGTC ACTCACAGGG CGGGCAGGTG 960

ACCAGGGCGG TGGAAGCGAC GCTGTGTCTT CCCAGCTGCC CTGCCTAGAG GCCCAGGGTG 1020

CAGGCACCGC CACCCACCCG TGTTCCCTAT CCAGGAGTGG ACCCACATCA CCCTATACTA 1080

CTTCCATC 1088

What is claimed:

1. A purified and isolated polynucleotide encoding human lysophosphatidic acid acyltransferase-1.

2. The polynucleotide of claim 1 wherein said polynucleotide is a DNA.

3. The polynucleotide of claim 1 wherein said polynucleotide is selected from the group consisting of a genomic DNA, a cDNA, and a chemically synthesized DNA.

4. The polynucleotide of claim 2 comprising the DNA sequence set out in SEQ ID NO: 1.

5. A polynucleotide encoding a polypeptide having lysophosphatidic acid acyltransferase-1 activity wherein said polynucleotide hybridizes under stringent hybridization conditions to the polynucleotide of SEQ ID NO: 1.

6. The polynucleotide of claim 1 wherein said polynucleotide is an RNA.

7. A vector comprising a DNA according to claim 2.

8. The vector of claim 7 wherein said DNA is operatively linked to an expression control DNA sequence.

9. A host cell stably transformed or transfected with a DNA according to claim 2.

10. A method for producing human lysophosphatidic acid acyltransferase-1 comprising the steps of growing a host cell according to claim 9 in a suitable nutrient medium and isolating the expressed polypeptide from the cell or the nutrient medium.

11. A purified and isolated polypeptide comprising the human lysophosphatidic acid acyltransferase-1 amino acid sequence of SEQ ID NO: 2.

12. An antibody substance specifically immunoreactive with human lysophosphatidic acid acyltransferase-1.

13. The antibody substance of claim 12 wherein said antibody is a monoclonal antibody.

14. A hybridoma cell line producing the monoclonal antibody of claim 13.

15. A humanized antibody according to claim 12.

16. A method of identifying a compound that is a modulator of human lysophosphatidic acid acyltransferase-1 comprising the steps of:

a) determining the acyl transferase activity of lysophosphatidic acid acyltransferase in the absence and presence of the compound;

b) comparing the acyl transferase activities observed in step (a); and

c) identifying the compound as a modulator of lysophosphatidic acid acyltransferase wherein a difference in acyl transferase activity is observed in the presence and absence of said compound.

17. A method for determining the presence of lysophosphatidic acid acyltransferase in a biological sample comprising the steps of:

a) exposing a human lysophosphatidic acid acyltransferase specific antibody to a biological sample; and

b) detecting the binding of the antibody to lysophosphatidic acid acyltransferase in the biological sample.

18. A method for detecting the presence of lysophosphatidic acid in a biological sample comprising the steps of exposing said biological sample to LPAAT-1 and radiolabeled acyl chain donor and detecting the formation of radiolabeled phosphatidic acid.

19. A diagnostic reagent comprising a detectably labeled polynucleotide encoding part or all of the human lysophosphatidic acid acyltransferase-1 amino acid sequences set out in SEQ ID NO: 2.

20. A composition comprising human lysophosphatidic acid acyltransferase-1 and a diluent, adjuvant or carrier.


Human
C. elegans
H. influenza
S. cerevisiae
E. coli
S. typhimurium
L. douglasii
C. nucifera

FIGURE 1

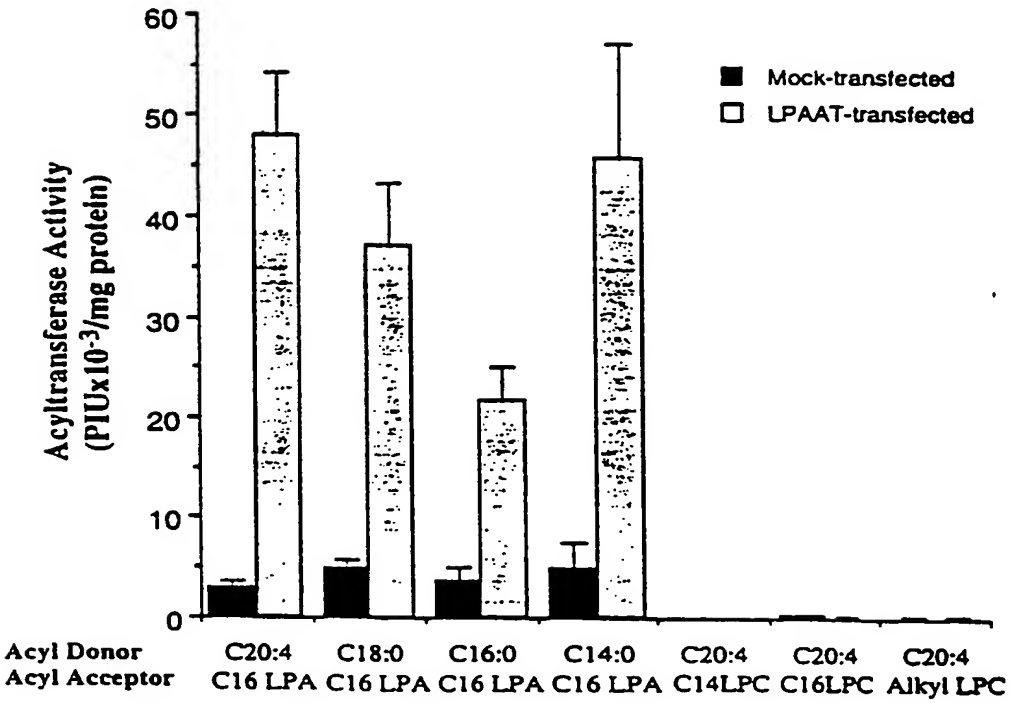
Acyltransferase Activity
(PIUx10-3/mg protein)
60
50
40
30
20
10
0
Mock-transfected
LPAAT-transfected
Acyl Donor
Acyl Acceptor
C20:4 C16 LPA
C18:0 C16 LPA
C16:0 C16 LPA
C14:0 C16 LPA
C20:4 C14LPC
C20:4 C16LPC
C20:4 Alkyl LPC

FIGURE 2

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